Open Literature Review

1. **Chemical Names:** Clothianidin, Imidacloprid

2. **PC Codes:** 044309, 129099

3. **CAS Nos.** 210880-92-5, 138261-41-3

4. **MRID:** None

5. **ECOTOX Record Number and Citation:**
Scholer, J. and V. Krischik. 2014. Chronic Exposure of Imidacloprid and Clothianidin Reduce Queen Survival, Foraging, and Nectar Storing in Colonies of *Bombus impatiens*. *PLOS ONE*. 9(3): e91573.

6. **Purpose of Review:** Clothianidin and Imidacloprid re-evaluation for pollinators

7. **Date of Review:** 1/28/15

8. **Description of Use:** Supplemental--Qualitative

9. **Summary of Study Findings:**

Executive Summary

This study examined bumble bee colonies exposed for 11 weeks to clothianidin or imidacloprid concentrations in sugar syrup (50%). Nominal concentrations were 0 (control), 10, 20, 50, and 100 ppb a.i. for both chemicals. Residues were analyzed in sugar syrup at only one time step for each experiment (2 experiments each for clothianidin and imidacloprid) during the study. Measured residues in the imidacloprid experiments were often not within 30% of nominal levels, but were usually closer to nominal in the clothianidin experiments. Data was presented as combined results from each experiment for imidacloprid and clothianidin. Queen mortality was significantly higher in the nominal 50 and 100 ppb treatment groups at 6 weeks exposure and in all treatment groups except for the 10 ppb treatment groups after 11 weeks of exposure compared to controls. Additional endpoints adversely affected at the 20 ppb concentration included worker movement, colony consumption and colony weight. Generally, statistically significant effects were not observed at the 10 ppb concentration in either the set of experiments, but for some endpoints (*e.g.* worker movement), inhibitions at the 10 ppb level were near 30% compared to controls. At 10 ppb imidiacloprid fewer drones were reported to be produced by workers, but this endpoint was not affected in the clothianidin treatments until 50 ppb.

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Methods

Commercially reared bumble bee (*Bombus impatiens*) colonies consisting of a queen and 30-50 workers (approximately 1 month old) were obtained from Koppert Biological Systems (Howell, Michigan) and fed Bee Happy sugar syrup (also from Koppert Biological Systems). Colonies were assessed for presence of queen and number of workers and weighed prior to study initiation. Bees and nest were subsequently placed into a brood box (21.6 x 17.8 x 0.6 cm) modified with a Plexiglas lid to permit weekly assessment using digital photography. The brood box was connected to a 29 cm^2 "flight box" by a plastic tube. Colonies were placed on benches in a greenhouse with temperature maintained at 22^0C and relative humidity of 60%.

Supplemental pollen that had been collected from pollen traps on honey bee colonies from the University of Minnesota in 2010 and stored frozen was mixed with Bee Happy sugar syrup to create a "pollen roll" coated with bees wax and was added every week to the floor of each brood box. 50% sugar syrup solutions were available in the flight box and were replaced three times per week. Prior to the exposure period, bees were fed untreated sugar syrup in the flight box for two weeks.

4 colonies were used per treatment and control at two different 11-week time periods for each compound (imidacloprid, July 6—September 15, 2011 and September 14, to November 23, 2011; clothianidin, January 18 to March 30, 2012 and March 12 to May 25, 2012). The study authors combined colony analysis of the two different treatment periods for each chemical into one analysis per chemical (*i.e.* each treatment group and control was considered by the study authors to have 8 colonies).

Commented [WM2]: Other than one of the clothianidin controls which must have had 5 colonies.

Queen status (presence, mortality) was recorded once per week. On weeks 4 and 8, activity within each colony's brood box was recorded using video twice for 30 minute intervals. The movement of queens and five workers per colony were quantified over a 300 second period by counting the number of seconds each bee moved in this time period. Bees were only used if they stayed in the video frame for the entire 300 second interval. The study authors did not report how they determined the 300 second interval from the two 30 minute sections of video that were obtained in these weeks nor how the five observed workers were chosen for observation.

Syrup consumption in the flight box was measured three times a week for each colony. Individual bee consumption was estimated by dividing the mean weekly consumption for each colony by the estimated number of bees in the nest. Weekly digital photographs were taken of each colony and analyzed for the number of wax pots with sugar syrup and number of bees in the next. Colony weights were recorded at the time the queen died or on week 11 at which time the number of wax pots containing sugar syrup was counted and weighted. Brood (eggs, larvae and pupae) were counted and described as dead or alive based on color. On weeks 4,6 and 8, bee weight was measured by removing 20 foragers from the flight box of each colony and

Commented [MWagman3]: I assume this was only done at week 11 or when the queen died and not weekly?

Commented [MWagman4]: How picked?

individually weighing each one prior to replacing them (marked, to ensure they were not reweighed in succeeding weight measurements) in the weight box. Dead bees were removed from the flight box every other week.

Stock solutions of 100,000 ppb a.i. imidacloprid (purity 99.5%) and clothianidin (purity 98.4%) were made from technical grade standards and dilutions of the stock solution mixed with 50% sugar syrup created the final nominal concentrations of 0, 10, 20, 50 and 100 ppb a.i. Stock solutions were made every 3 weeks and the sugar syrup solutions were made weekly.

Sugar syrup was analyzed for chemical residues at one date during the middle of each tests' exposure period (*i.e.*. for imidacloprid, analysis was conducted in August and October, 2011 while for clothianidin analysis was conducted in March and April, 2012). Samples of the pollen (n=8) used to make the pollen rolls were also taken for residue analysis. On 3 dates per test (one from the first treatment period and two from the second treatment period), colony stored sugar syrup in wax pots was combined from 3 different colonies for each treatment (not controls?) and analyzed. Stock, sugar syrup samples and pollen samples were stored at -80^{0}C and analyzed at USDA, AMS in Gastonia, NC. Residue analysis was conducted for parent imidacloprid and clothianidin, metabolites and 4 fungicides: carboxin, metalaxyl, tebuconazole, and trifloxystrobin.

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Commented [MWagman6]: Study authors said all metabolites of parent imi and clothi, but did not list which ones anywhere in the report.

Statistics

Anayses were performed using ProcMixed (SAS Institute, 2010) and JMP Pro 9.0.2 (SAS Institute, 2010). Cumulative queen mortality, worker movement and the number of wax sugar syrup pots were analyzed using Kruskal-Wallis nonparametric Chi-square tests and Wilcoxon nonparametric multiple comparison test. Colony and individual bee food consumption, number of bees, colony and individual bee weight, wax syrup pot weight, brood production and bee caste production were tested for equal variances using Levine's, transformed if needed and assessed with either Tukey-Kramer Multiple range test (MRT), ANOVA and Tukey-Kramer MRT or, if data still having non-homogenous variances after transformation, Welch's test. Where significant interactions were seen in ProcMixed, the data was subsequently analyzed with ANOVA's for all treatments by week.

Results

Out of the 8 pollen samples taken from pollen traps from honey bee colonies, 7 samples were reported to have residues of clothianidin, imidacloprid, neonicotinoid metabolites and the four fungicides below the level of detection (LOD). The remaining pollen sample had measured residues of 4 ppb a.i. imidacloprid. Residues of neonicotinoid metabolites and fungicides were below the LOD in all treatment and stock solution samples. Stock solution (nominal 100,000 ppb a.i.) residues were reported to be slightly higher than nominal concentrations (imidacloprid,

Commented [MWagman7]: LOD and LOQ not reported.

13% greater, and clothianidin, 3% greater). In imidacloprid sugar syrup samples (2 samples/treatment**, Table 1**, reproduced from Scholer and Krischik, 2014), 3 of the 8 samples from the two experiments had substantially (> 30% difference) higher measured residues than the nominal treatments (one sample each from the 10 ppb, 50 ppb and 100 ppb treatments) with one sample substantially lower than nominal (20 ppb). The measured concentrations in the 10 ppb and 20 ppb imidacloprid treatment groups overlap, with higher residues measured in the nominal 10 ppb treatment group than were found in the nominal 20 ppb treatment group during the 2nd experiment. In clothianidin sugar syrup samples two samples from across the two experiments had substantially lower residues than the nominal treatments (50 ppb, 100 ppb).

Samples of sugar syrup stored in wax pots also had no detectable neonicotinoid metabolites or fungicides. Measured residues in wax pots for the 20 ppb—100 ppb a.i. treatments were considerably lower (-45% to -100%) than nominal treatment levels. The study authors also reported that mean residues in stored syrup from wax pots was also substantially (≥ 35%) lower in these treatments compared to the measured residues in the sugar syrup samples.

Commented [MWagman8]: Though, their analysis here is slightly flawed as they measured residues in sugar syrup at 2 time points, but measured residues in wax pots at 3 time points and averaged those 3 equally which biases the samples towards the residues from the 2nd treatment period.

Queens were not observed in the flight box, so the study authors' assumed that queens fed on sugar syrup stored in wax pots. Acute effects were not observed from any of the treatments on queen bees. At week 6, queen mortality was significantly higher in both chemicals' 50—100 ppb a.i. treatments compared to control queens (**Figure 1**, reproduced from Scholer and Krischik, 2014). At the end of the exposure period, week 11, queen mortality was significantly higher for all treatments over 10 ppb a.i. compared to controls. No significant differences were observed in queen movement between the different treatments and controls.

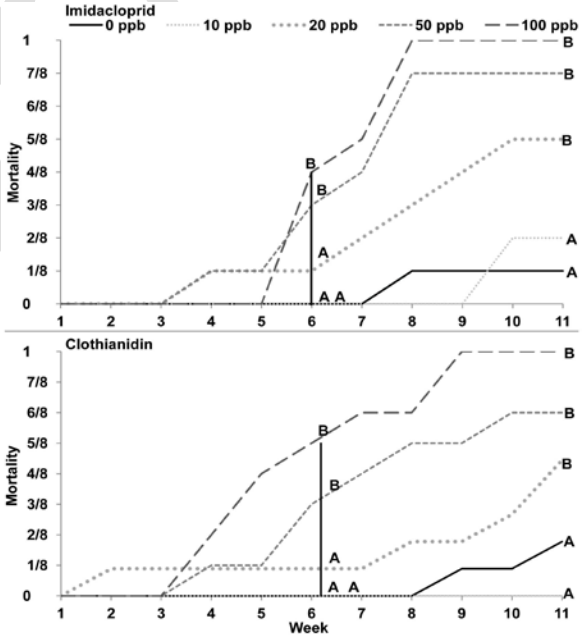


Figure 1. Queen mortality from Weeks 1—11 for Colonies Exposed to Varying Doses of Imidacloprid or Clothianidin. Different letters denote significant differences between treatments and controls. Imidacloprid, Week 6: Chi-square test=9.26, DF=4, 235, p<0.055; week 11: Chi-square test = 75.49, DF = 4,435, p<0.001. Clothianidin, Week 6: Chi-square test =22.87, DF=4, 247, p<0.001, week 11: Chi-square test = 102.78, DF=4, 457, p<0.001.

Table 1. Imidacloprid and Clothianidn Residues in Sugar Syrup Stock Solutions (50%) and Treatments from One Sample in Each Treatment Period and from Stored Syrup in Wax Pots (3 colonies mixed) from Treatment Period 1 (1 sample) and Treatment Period 2 (2 samples) Experiments. Residue determined using USDA methodology at USDA, AMS, Gastonia, NC.

Imidacloprid										
	Residue in sugar syrup				**Residues in stored syrup from wax pots (measured at the end of each treatment period)**					
Nominal (ppb)	**Aug-11**	**Oct-11**	**Mean Conc.**	**Differnce Mean and Nominal**	**Sep-11**	**Nov-11**	**Nov-11**	**Mean Conc.**	**Difference Mean and Nominal**	**Difference Wax Pots and Measured Sugar Syrup Residues**
0	0	0	0	0%	0	0	0	0	0%	0%
10	10	17	14	40%	11	8	15	11	10%	-22%
20	20	11	16	-20%	6	11	6	8	-60%	-50%
50	80	61	71	42%	60	0	0	29	-40%	-72%
100	114	139	127	27%	3	0	No sample	1	-99%	-100%
100,000	107,000	118,000	112,500	13%	-	-	-	-	-	-
Clothianidin										
	Residue in sugar syrup				**Residues in stored syrup from wax pots (measured at the end of each treatment period)**					
Nominal (ppb)	**Aug-11**	**Oct-11**	**Mean Conc.**	**Differnce Mean and Nominal**	**Sep-11**	**Nov-11**	**Nov-11**	**Mean Conc.**	**Difference Mean and Nominal**	**Difference Wax Pots and Measured Sugar Syrup Residues**
0	0	0	0	0%	0	0	0	0	0%	0%
10	8	10	9	-10%	8	6	9	8	-20%	-12%
20	14	20	17	-15%	10	11	12	11	-45%	-35%
50	34	43	39	-22%	0	0	0	0	-100%	-100%
100	67	85	76	-24%	0	0	0	0	-100%	-100%
100,000	98,800	110,000	103,400	3%	-	-	-	-	-	-

For worker bee movement, the study authors' removed both 100 ppb a.i. treatments from the analysis as there were too few bees to quantify movement. For both imidiacloprid and clothianidin experiments, bees in the control groups moved significantly more than those in all treatment groups greater than 10 ppb a.i. (inhibitions ranged from 29%--59% for imidacloprid and 30%--73% for clothianidin treatment groups). At nominal 20 ppb a.i. imidacloprid and clothianidin, worker movement was inhibited 47% and 32%, respectively. Worker movement showed a monotonically decreasing dose-response trend through all treatment groups. Although not statistically significant, worker movement activity was 29% and 30% lower in the 10 ppb a.i. imidacloprid and clothianidin groups, respectively compared to workers in the control groups.

Commented [MWagman9]: The study authors described this as "faster", but as far as I could tell from their M/M section, they were measuring duration of movement or forager activity, not speed.

Colony consumption for both a.i. showed a significant interaction between week and treatment **(Figure 2** and **Table 2**, reproduced from Scholer and Krischik, Tukey-Kramer analysis) and were then post-hoc analyzed with ANOVA and Tukey-Kramer. Significantly more sugar syrup was consumed in weeks 2, 6, and 8 in the control groups than compared to all treatment groups for both a.i. At week 4, significantly more sugar syrup was also consumed in the control group compared to all the clothianidin treatment groups while for imidacloprid, significantly more sugar syrup was consumed in both the 0 and 10 ppb a.i. imidacloprid groups compared to the higher imidacloprid treatments. At week 2, inhibition of colony consumption of sugar syrup solution compared to controls in the 10 ppb a.i. groups was 32% and 26%, respectively for imidacloprid and clothianidin. By week 8, inhibition of sugar syrup solution consumption compared to controls in the 10 ppb a.i. groups was 50% and 40%, respectively for imidacloprid and clothianidin.

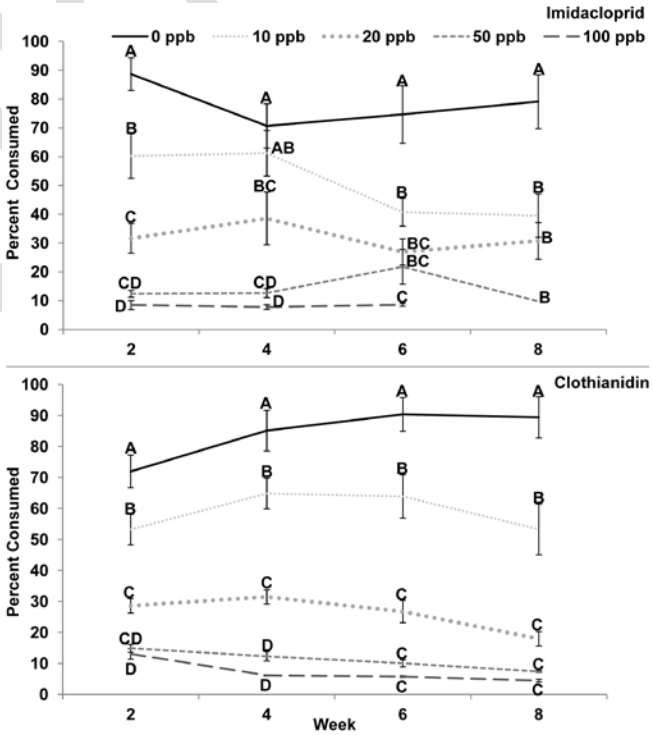


Figure 2. Colony Sugar Syrup Consumption in Weeks 2, 4, 6 and 8. Imidacloprid, Week 2: F=52.51, DF=4, 16, p<0.001, Week 4:F=27.40, DF=4, 14, p<0.001, Week 6: F=22.61, DF=4, 12, p<0.001, Week 8: F=7.67, DF=3,17, p=0.002. Clothianidin, Week 2:F=42.05, DF=4, 17, p<0.001, Week 4: F=91.96, DF=4,14, p<0.001, Week 6: F=42.77, DF=4, 28, p<0.001, Week 8: F=48.52, =4, 8, p<0.001. ANOVA, Tukey-Kramer MRT by treatment for each week are on the figures, Table 2.

Table 2. Statistical Analysis for Multiple Parameters. When a week effect in ProcMixed is significant, the Tukey-Kramer MRT is on the figure and the statistics are on this table. When a treatment effect in ProcMixed is significant, the statistics, mean, SE, and Tukey-Kramer MRT for each treatment is on this table (SAS, 2010). When an interaction effect is significant in ProcMixed, the statistics are on this table. Then the data were analyzed individually by week for treatment and the statistics are on the figure legend (ANOVA, Tukey-Kramer MRT, SAS, JMP, 2010). Treatment effects are presented for each treatment as Mean ± SE. Letters in the Interaction Effect column represent differences in significance relative to controls.

Figure	Parameter	Wk/trt	Week effect F (df), P	Treatment effectF (df), P	% Inhibition Relative to Control	Interaction effectF (df), P
Imidacloprid						
2	**Colony consumption**	2, 4, 6, 8	1.91 (3,77), 0.1356	32.4 (4,35), 0.0001	N/A	2.35 (11,77), 0.0148
		Nominal Trt (ppb)	**n**	**Percent Provided Syrup Consumed**	**--**	**Tukey-Kramer**
		0	31	0.78±0.05	--	A
		10	32	0.50±0.05	36%	B
		20	27	0.30±0.05	62%	C
		50	21	0.15±0.06	81%	C
		100	20	-[1]	--	-
3	**Bee Consumption** week effect on figure	2, 4, 6, 8	8.52 (3,76), 0.0001	1.59 (4,35), 0.1998	N/A	0.87 (11,76), 0.5698
		Nominal Trt (ppb)	**n**	**Consumption/bee (mL)**	**--**	**Tukey-Kramer**
		0	31	1.56±0.23	--	A
		10	32	1.13±0.25	28%	A
		20	27	0.91±0.23	42%	A
		50	21	0.61±0.37	61%	A
		100	19	-	--	-
8	**Bees on nest** week effect on figure	0, 2, 4, 6, 8	21.4(4,112), 0.0001	3.67 (4,35), 0.0135	N/A	1.34 (15,112), 0.1910
		Nominal Trt (ppb)	**n**	**Number of bees on nest**	**--**	**Tukey-Kramer**
		0	39	50.49±10.05	--	A
		10	40	41.90±9.39	17%	A
		20	35	36.91±7.60	27%	A
		50	29	39.07±8.37	23%	A
		100	28	26.04±6.83	48%	-
N/A	**Bee weight**	4, 6, 8	8.76 (2,38), 0.0007	2.20 (4,35), 0.0894	N/A	0.41 (8,38), 0.9096
		Nominal Trt (ppb)	**n**	**Weight (Units not reported)**	**--**	**Tukey-Kramer**
		0	21	0.14±0.0083	--	A
		10	21	0.14±0.0083	0%	A

Commented [MWagman10]: Table is from the supplementary materials for the article and I have added a % inhibition column and provided the relevant titles and units where possible.

Commented [MWagman11]: Significant?

		20	16	0.12±0.0095	14%	A
		50	16	0.12±0.0095	14%	A
		100	14	0.11±0.010	21%	A
Clothianidin						
2	**Colony consumption**	2, 4, 6, 8	1.72 (3,85), 0.1689	85.7 (4,36) 0.0001	N/A	2.76 (12,85), 0.0032
		Nominal Trt (ppb)	**n**	**Percent Provided Syrup Consumed**	**--**	**Tukey-Kramer**
		0	36	84.21±3.27	--	A
		10	32	58.81±3.47	30%	B
		20	31	26.30±3.50	69%	C
		50	23	10.46±3.87	88%	D
		100	19	7.04±4.29	92%	D
3	**Individual Bee consumption, week effect on figure**	2, 4, 6, 8	3.53 (3,84), 0.0183	14.13(4,36), 0.0001	N/A	0.96 (12,84), 0.4918
		Nominal Trt (ppb)	**n**	**Consumption/bee (mL)**	**--**	**Tukey-Kramer**
		0	36	1.13±0.094	--	A
		10	31	0.73±0.10	35%	B
		20	31	0.44±0.10	61%	BC
		50	23	0.25±0.11	78%	C
		100	19	0.19±0.12	83%	C
8	**Bees on nest** week effect on figure	0, 2, 4, 6, 8	26.9(4,120), 0.0001	2.95 (4,37), 0.0328	N/A	3.99(16,120), 0.0001
		Nominal Trt (ppb)	**n**	**Number of bees on nest**	**--**	**Tukey-Kramer**
		0	45	69.96±6.62	--	A
		10	39	70.45±7.04	-1%	A
		20	40	55.56±6.79	21%	A
		50	31	47.78±7.37	32%	A
		100	27	43.77±7.75	37%	A
Results section	**Bee weight**	4, 6, 8	4.53 (2,46), 0.0161	5.58 (4,34), 0.0015	N/A	1.96 (7,46), 0.0807
		Nominal Trt (ppb)	**n**	**Weight (Units not reported)**	**--**	**Tukey-Kramer**
		0	27	0.12±0.69	--	A
		10	24	0.13±0.75	-8%	AB
		20	23	0.15±0.91	-25%	B
		50	13	0.16±0.70	-33%	AB
		100	7	0.11±0.56	8%	-

[1] The 100 ppb a.i. group had no consumption after week 6 (**Figure 2**).

Individual bee consumption was not significantly different between control and all imidacloprid treatment groups (inhibitions ranged from 28%--61%, **Figure 3** and **Tables 2,** both reproduced from Scholer and Krischik, 2014). However, for clothianidin, significant differences in individual bee consumption were observed for all treatment groups compared to controls (inhibitions ranged from 35%--83%). When individual bee consumption was analyzed

Commented [MWagman12]: Significance?

Commented [MWagman13]: I admit to being confused on this. The table above (adapted from Table S1 in the study) seems to show that there were significant differences for all clothi treatment groups compared to controls, however the text of Figure 3 in the study specifically says that"ProcMixed did not show a significant interaction for imidacloprid or clothianidin, Table S1"

individually by week, the study authors reported that in week 2, there was significantly more sugar syrup consumption in control groups than in imidaclorpid treatment groups (≥ 50% inhibition in all treatments, **Table 3**, modified from data from Scholer and Krischik, 2014) while in the clothianidin experiments during week 2, there were no significant differences between the 0 and 10 ppb treatments, but all treatment groups over 10 ppb were significantly inhibited (>60% inhibition in these treatments) compared to controls. Week 4 treatments showed significantly more sugar syrup consumed by individual bees in the control groups compared to all imidacloprid (≥40% inhibition) and clothianidin (>50% inhibition) treatments over 10 ppb. Week 6 and 8 had no significant differences between individual bee consumption for control and any imidacloprid treatments. Similarly, clothianidin treatments showed no significant differences between individual bee consumption in control and treatment groups in week 8, but clothianidin treatments greater than 10 ppb were significantly inhibited (≥59%) in week 6.

Figure 3. Individual Bee Consumption. ANOVA, Tukey-Kramer MRT by treatment for each week are on the figures to compare the two chemicals, but ProcMixed did not show a significant interaction for imidacloprid or clothianidin (**Table 2**).

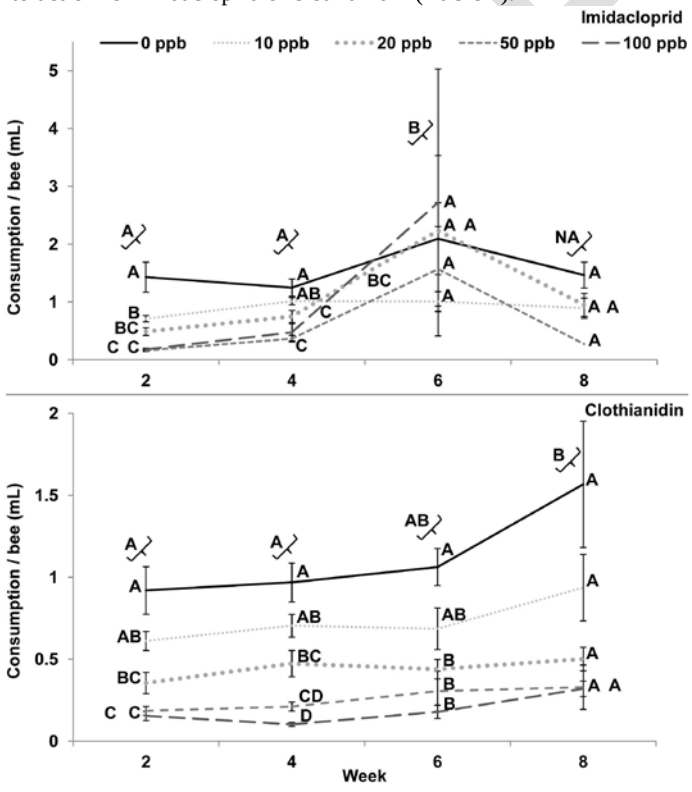


Table 3. Individual Bee Consumption in ml and ng by Treatment for Each Week. Imidacloprid, Week 2: F = 30.97, DF = 4, 16, p<0.001, Week 4: F = 10.31, DF = 4, 33, p<0.001, Week 6: F = 0.89, DF = 4, 8, p = 0.513, Week 8: F = 2.51, DF = 3, 17, p = 0.093, Clothianidin, Week 2: F = 17.68, DF = 4, 17, p<0.001, Week 4: F = 32.73, DF = 4, 15, p<0.001, Week 6: F = 9.37, DF = 4, 28, p<0.001, Week 8: F = 4.32, DF = 4, 8, p = 0.035, ANOVA, Tukey-Kramer MRT by treatment for each week.

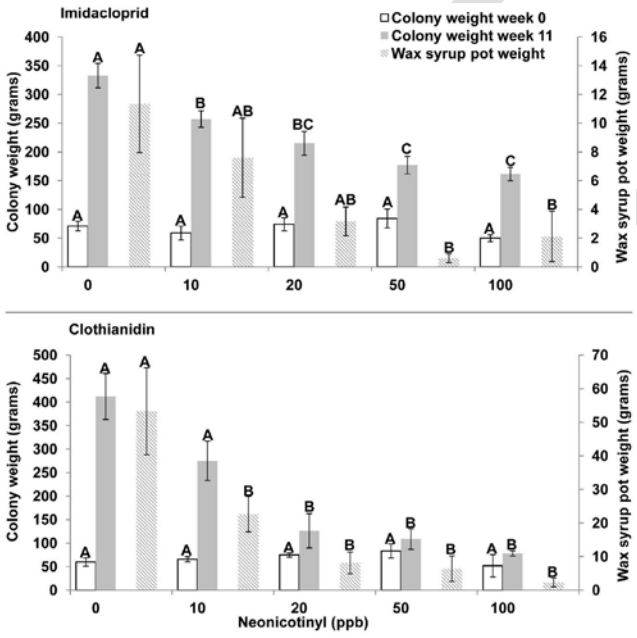
Imidacloprid trt*	week	No bees	No colonies	mean ml consumed	% Inhibition	± SE	ng consumed** trt (residue)
0 (0)	**2**	61	8	1.42999	--	0.25893	0 (0)
10 (14)	2	65	8	0.70978	50%	0.05295	7.1 (9.9)
20 (16)	2	54	8	0.48825	66%	0.06616	9.6 (7.7)
50 (71)	2	62	8	0.16106	89%	0.01713	8 .0 (11.4)
100 (127)	2	35	8	0.17845	88%	0.02197	17.8 (21.6)
0 (0)0	**4**	49	8	1.24777	--	0.14967	0 (0)
10 (14)	4	46	8	1.01269	19%	0.0628	10.1 (14.1)
20 (16)	4	37	7	0.74858	40%	0.10279	15.0 (11.8)
50 (71)	4	29	7	0.3655	71%	0.05838	18.3 (25.6)
100 (127)	4	19	8	0.47517	62%	0.14935	47.5 (59.7)
0 (0)	**6**	41	8	2.09384		0.6222	0 (0)
10 (14)	6	33	8	1.00837	52%	0.1705	10.1 (14.0)
20 (16)	6	23	7	2.23146	-7%	1.3026	4.5 (35.7)
50 (71)	6	18	5	1.5684	25%	0.7354	7.8 (110.8)
100 (127)	6	9	3	2.72029	-30%	2.3082	272.0 (345.4)
0 (0)0	**8**	46	7	1.46522		0.22418	0 (0)
10 (14)	8	34	8	0.89297	39%	0.17523	8.9 (11.1)
20 (16)	8	28	5	0.94857	35%	0.20027	19.0 (15.0)
50 (71)	8	27	1	0.2716	81%	-	13.6 (19.2)
100 (127)	8	0	0	-	--	-	-

Clothianidin trt*	week	No bees	No colonies	mean ml consumed	% Inhibition	± SE	ng consumed** trt (residue)
0 (0)	2	67	9	0.919682	--	0.14505	0 (0)
10 (9)	2	67	8	0.611672	33%	0.05839	6.1 (5.5)
20 (17)	2	70	8	0.35474	61%	0.06508	7.0 (6.0)
50 (39)	2	64	8	0.185079	80%	0.02668	9.0 (7.0)
100 (76)	2	74	8	0.154299	83%	0.02834	15 (11.4)
0 (0)	4	75	9	0.96903	--	0.11866	0 (0)
10 (9)	4	73	8	0.704457	27%	0.06912	7.0 (6.3)
20 (17)	4	58	8	0.47325	51%	0.08005	9.5 (8.0)
50 (39)	4	47	7	0.211047	78%	0.02728	10.6 (8.2)
100 (76)	4	47	6	0.102446	89%	0.01239	10.2 (7.6)
0 (0)	6	72	9	1.06257	--	0.11362	0 (0)
10 (9)	6	79	8	0.68567	35%	0.12723	6.9 (6.1)
20 (17)	6	52	8	0.43893	59%	0.05931	8.8 (7.3)
50 (39)	6	34	5	0.3053	71%	0.12282	15.3 (11.7)
100 (76)	6	23	3	0.17881	83%	0.0406	17.8 (12.9)
0 (0)	8	63	9	1.56736	--	0.38544	0 (0)
10 (9)	8	47	7	0.93701	40%	0.20287	9.4 (8.4)
20 (17)	8	33	7	0.50118	68%	0.07208	10.0 (8.5)
50 (39)	8	22	3	0.32934	79%	0.13535	16.5 (12.5)
100 (76)	8	11	2	0.3197	80%	0.04697	31.9 (23.6)

Commented [MWagman14]: This is modified from Table S2 in the study by lining up the treatments by week and calculating % inhibitions per week. In a few cases my calculated % inhibitions were very slightly different from the study authors (*e.g.* Week 4 Imidacloprid 20 ppb is calculated here as 40% difference while the study authors reported it as 42% difference).

At week 0, there were no significant differences in colony weight for controls and any imidacloprid or clothianidin treatment (**Figure 4**, reproduced from Scholer and Krischik, 2014). At week 11 colony weight was reported to be significantly greater in controls compared to all imidacloprid (inhibition 23—51%) or clothianidin treatments greater than 10 ppb (69—81% inhibition). Although not statistically significantly different from controls, the 10 ppb a.i. clothianidin treatment group was reported to weigh 33% less (275 g compared to 412 g) compared to control colonies.

Figure 4. Colony Weight and Syrup Weight in Wax Pots. Imidacloprid, colony weight, Week 0: F=1.84, DF=4, 16, p=0.170, Week 11: F=16.20, DF=4, 35, p<0.001; syrup weight, Week 11: F=4.83, DF=4, 15, p=0.011. Clothianidin, colony weight, Week 0: F=0.87, DF=4, 37, p=0.492, Week 11: F=16.10, DF=4, 37, p<0.001; syrup weight Week 11: F=6.83, DF=4, 16, p = 0.002, ANOVA, Tukey-Kramer MRT.

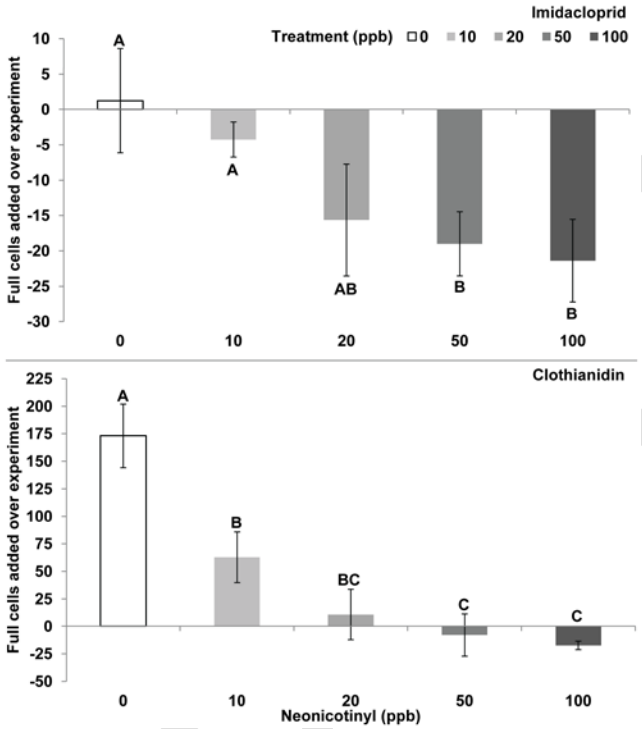


The weight of stored syrup in wax pots was significantly reduced in imidacloprid treatments greater than 20 ppb (inhibition ranged from 33% at 10 ppb to 91% at 100 ppb, **Figure 4**) compared to controls, but was significantly reduced in all clothianidin treatments (>57% inhibition).

Control performance for the number of wax pots was markedly different between the two sets of experiments. Controls in the imidacloprid experiments started with mean wax pots of 21 pots and gained an average of 1.2 pots through the 11 weeks while controls in the clothianidin experiments started with 36 pots and gained an additional 173 throughout the experiments (**Figure 5**, reproduced from Scholer and Krischik, 2014). Imidacloprid treatments had significantly fewer wax pots gained only for treatments greater than 20 ppb (≥ 2,000% inhibition for these treatment levels), while wax pots gained was significantly reduced in all clothianidin treatments (64—110% inhibition). The study authors pointed to the declining measured residues in the sugar syrup from the wax pots as

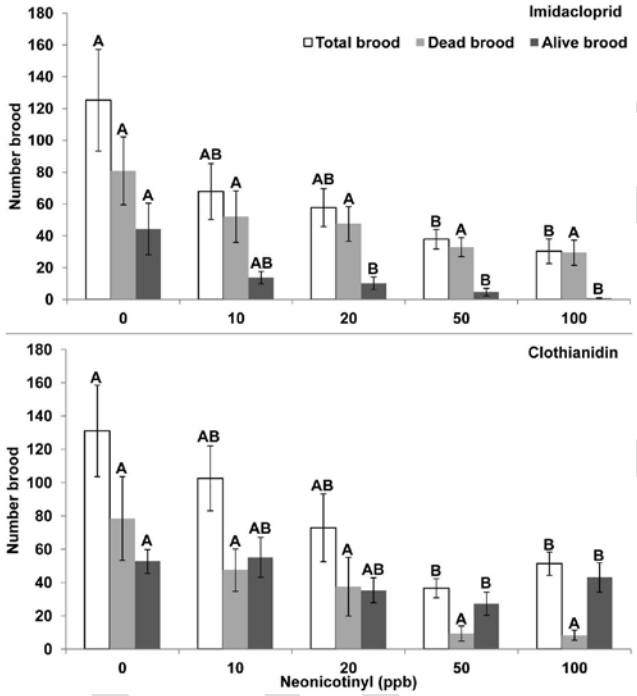
additional evidence that sugar syrup was not being stored in the nominally higher (> 20 ppb a.i.) imidacloprid and clothianidin treatments.

Figure 5. Wax Syrup Pots Added Through the 11 Week Exposure Period. Imidacloprid, Chi-square test = 10.23, DF =4, p = 0.0368. Clothianidin, Chi-square test, F=21.54, DF=4, p<0.0002, Krukal-Wallis, Wilcoxon Test.



No significant differences were observed in the treatments compared to the controls for the number of dead brood in either the imidacloprid or the clothianidin experiments. However, the number of live brood at the end of the exposure period was significantly greater in controls compared to imidacloprid treatments > 10 ppb a.i. and clothianidin treatments > 20 ppb a.i. (**Figure 6**, reproduced from Scholer and Krischik, 2014). The study authors explained this as a function of queen mortality in those treatments, despite the observation that queen mortality in the clothianidin 20 ppb a.i. treatment groups was not significantly different from queen mortality in the control groups (**Figure 1**). Total brood (both live and dead) was reported to be significantly greater in control groups compared to both imidacloprid and clothianidin treatments > 20 ppb a.i. (**Figure 6**).

Figure 6. Total, Dead, and Alive Brood at the End of Exposure on Week 11. Imidacloprid, Week 11: Total Brood: F=2.99, DF=4, 17, p=0.049, Dead Brood: F=1.67, DF=4, 17, p=0.205, Alive Brood: F=5.74, DF=4, 14, p=0.006. Clothianidin, Week 11: total Brood: F=4.16, DF=4, 37, p=0.007, Dead Brood: F=1.83, DF=4, 37, p=0.144, Alive Brood: F=4.13, DF=4, 17, p=0.016, ANOVA, Tukey-Kramer MRT.



Production of workers and new queens was not statistically significantly different between either imidacloprid or clothianidin treatments and their respective controls (**Figure 7**, reproduced from Scholer and Krischik, 2014). However, while the maximum inhibition of daughter queen production in imidacloprid treated colonies was 28% at the 100 ppb treatment level, inhibitions ranged from 58% at 10 ppb clothianidin to 86% at 100 ppb clothianidin. Drone production was significantly decreased in all imidacloprid treatments (inhibitions ranging from 63%–97%) and in clothianidin treatments greater than 20 ppb a.i. (inhibitions ranging from 25% at 10 ppb to 97% at 100 ppb). Controls in the imidacloprid experiments had similar mean daughter queen production as clothianidin controls (5.7 compared to 7.4 new queens), but produced more than twice as many drones (135 compared to 64 drones).

The study authors reported that for the imidacloprid experiments, there were no differences in the total number of bees on the nest between controls and imidacloprid treatments, but when analyzed by week, there were significantly more bees on the nest in controls compared to the 100 ppb treatments at week 4 (inhibitions ranged from 4% at 10 ppb to 60% at 100 ppb) and week 6 (inhibitions ranged from 9% at 20 ppb to 79% at 100 ppb). The study authors reported that for clothianidin there was a significant interaction of week and treatment (**Figure 8** and **Table 2**, reproduced from Scholer and Krischik, 2014), but when analyzed by week, there were significantly more bees on nest during week 6 in controls compared to clothianidin treatments greater than 20 ppb (inhibitions ranged from -10% at 10 ppb to 68% at 100 ppb).

Commented [MWagman15]: I do not see this from either Figure 8 (supplemental Figure S1 in the original paper) or Table 2 and find this very confusing.

The study authors reported that bee weight was not significantly different between imidacloprid treatments (inhibitions ranging from 0%—21%, **Table 2**), but that clothianidin treatments were significantly different at the 20 ppb level compared to clothianidin controls (inhibitions ranging from -33% to 8%). However, at the 20 ppb clothianidin treatment level, bees actually weighed 25% more than bees in the controls; therefore, it is unclear whether this can be considered an adverse effect of treatment.

Figure 7. Worker, Male and Daughter Queen Production. Imidacloprid, Week 11: All Castes: F=4.62, DF=4, 35, p=0.004, Workers: F=1.92, DF=4, 35, p=0.129, Males: F=4.59, DF=4, 14, p=0.014, Queens: F=0.19, DF=4, 35, p=0.945. Clothianidin, Week 11: All Castes: F=5.12, DF=4, 37, p=0.002, Workers: F=2.15, DF=4, 37, p=0.094, Males: F=7.44, DF=4, 16, p=0.002, Queens: F=2.23, DF=4, 37, p=0.085, ANOVA, Tukey-Kramer MRT.

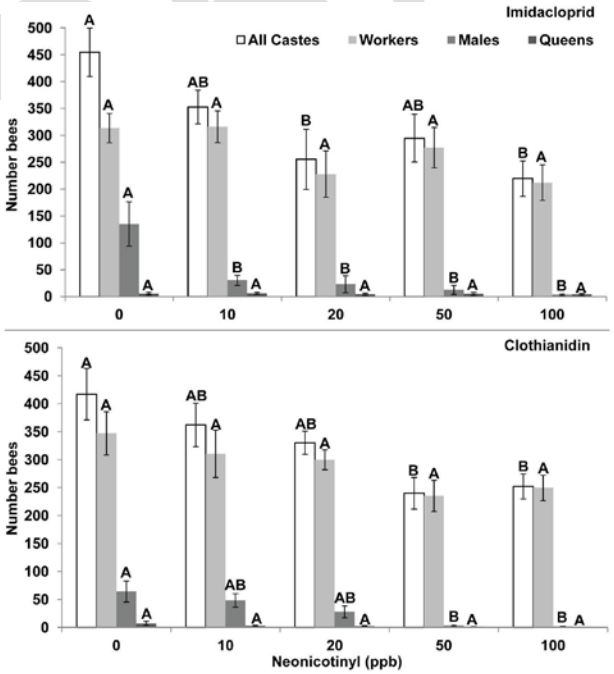
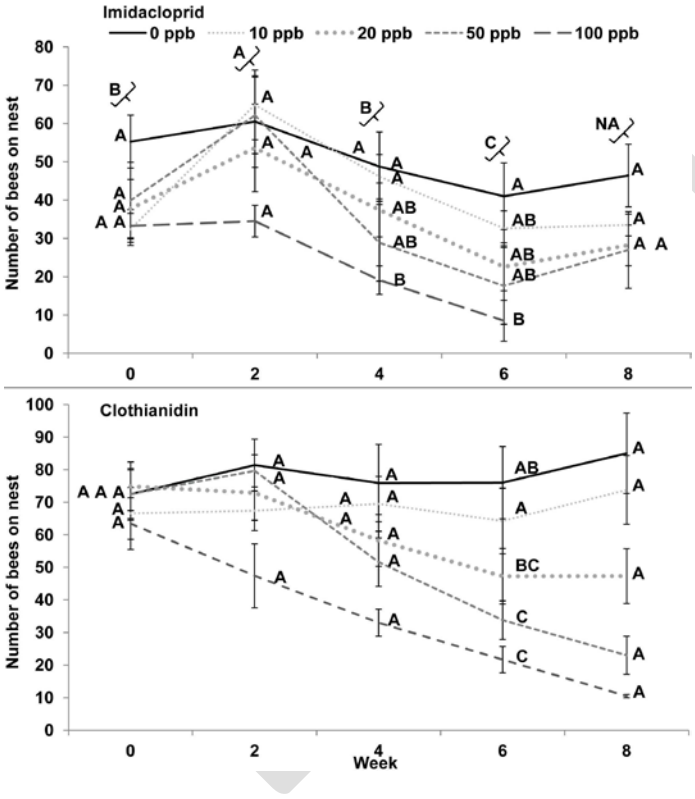


Figure 8. Total Number of Bees (Workers, Drones and Queens) on Nest. Imidacloprid, Week 0: F=2.55, DF=4, 35, p=0.057. Week 2: F=4.20, D=4, 17, p=0.016, Week 4: F=4.82, DF=4, 16, p=0.010, Week 6: F=3.84, DF=4, 12, p=0.031, Week 8: F=1.77, DF=3, 17, p=0.192. Clothianidin, Week 0: F=0.39, DF=4, 37, p=0.813, Week 2: F=0.21, DF=4, 36, p=0.928, Week 4: F=2.16, DF=4, 33, p=0.095, Week 6: F=4.52, DF=4, 28, p=0.006, Week 8: F=8.29, DF=4, 8, p=0.005. ANOVA, Tukey-Kramer MRT by treatment for each week are on the figures to compare the 2 chemicals, but ProcMixed did not show a significant interaction for imidacloprid, but did for clothianidin (**Table 2**)

Commented [MWagman16]: ? I see no evidence of this.



Data Quality Evaluation

The study authors in effect combined two experiments each for bumble bee colonies exposed to imidacloprid (11 weeks exposure each from July—September, 2011 and September—November, 2011) and clothianidin (11 weeks exposure each from January—March, 2012 and March—May, 2012) and combined the data from each experiment into one dataset for each chemical. Conducting experiments at different times and combining the data into one dataset for statistical analysis is not considered an appropriate methodology by EPA/PMRA. It is unclear from the

study report whether experimental conditions and/or control performance may have been different between each experiment for each chemical. Some studies will attempt to combine data from multiple experiments into one analysis by using an analysis of covariance (ANCOVA) to see if there is a statistical effect of "Experiment" on the analysis, however the study authors did not present the results of any such statistical analysis in this article. Control performance may have varied significantly by experiment; although the authors did not present individual experiment control results, it is worth comparing the large disparity in control performance for added wax pots between the imidacloprid and clothianidin results as an indication that control performance may have been dissimilar across the multiple experiments.

The study authors only took samples to analyze sugar syrup residues once per experiment and these samples (especially during the 2nd imidacloprid experiment) were frequently substantially different from nominal rates (*i.e.* 50% of measured imidacloprid samples were >30% different from nominal concentrations with 25% of measured clothianidin samples also being substantially different). The study authors reported that mean measured treatment residues did not overlap, however the range of measured values do overlap in the 2nd imidacloprid experiment between the 10 ppb and 20 ppb nominal treatment group (**Table 1**). This creates considerable uncertainty around the results for these concentrations which might have been somewhat alleviated had the authors provided the results of each experiment separately. Given the lack of adequate analytical sampling, the reviewer has reported the results in this DER using nominal concentrations.

Commented [WM17]: I was thinking of making a results table showing the nominal NOAEC/LOAEC for each endpoint (by chemical). I ended up not doing this due to the uncertainties raised around the actual exposures, but if PMRA thinks such a table would be useful, I'm not totally against it.

The reported mean residues in the stored syrup in wax pots at the experiment's end may be somewhat biased towards the 2nd experiment's results for each chemical as two analytical samples were taken in the 2nd experiments, while only a single sample was taken in the first experiments. However, as results are not reported using the wax pot stored sugar syrup concentrations for any endpoint, this is not considered to have a major effect on the validity of the study results.

10. Peer Review

Primary Reviewer Comments

Rationale for Use:

The study provides information on effects of imidacloprid and clothianidin in sugar solution (surrogate for nectar) fed to colonies of bumble bees over an 11 week exposure period. The study elucidates potential impacts from environmentally relevant field exposure concentrations on a number of colony-level endpoints including queen mortality, bumble bee movement, feed consumption, hive weight, stored honey and brood production.

Limitations of Study:
As described above in the Data Quality Evaluation section, there are major uncertainties in this study regarding how the study authors conducted their analytical sampling for residues and their statistical analysis combining the effects results from trials conducted at different times.

Both pollen and nectar food sources may be contaminated with neonicotinoids, whereas this study primarily considered the nectar exposure route (*i.e.* provided pollen was reported to have residues below the LOD in 87.5% of samples). If pollen is also contaminated with neonicotinoid residues, especially at levels similar to the higher nominal treatment concentrations in nectar from this study, than there may be potential for additional effects. Conversely, bumble bees were not free-foraging in this study and had no option but to consume treated nectar, whereas in actual field conditions, the diet may be more diverse and bees may avoid contaminated food sources.

Colonies were exposed to contaminated sugar syrup solutions for 11 weeks. In normal field conditions, colonies may not be exposed to the high levels of residues described in the higher tested treatments or even the lower residues tested in the lower treatments for such an extended period of time. Additionally, no post-exposure period was examined in this study to see if colonies may recover from exposures or if effects on queens may persist even subsequent to overwintering.

Description of Use in Document:
Qualitative. The study may be used in risk assessments to characterize the effects of sub-lethal exposures on a wide range of colony endpoints to bumble bees. Uncertainties regarding both the actual residues colonies were subjected to and potential variation from differing conditions between the experiments conducted at different times prevent quantitative use of the study. Nevertheless, the study results provide sufficient information to indicate high potential for risk to bumble bee colonies when residues are high (>20 ppb) for extended periods of time. Potential for risk to bumble bee colonies remains uncertain at lower doses (10-20 ppb) and the study provides little information on potential effects where residues may be quite low (<10 ppb).

Secondary Reviewer Comments:
[Provide any comments from secondary reviewer. Comments should be high level (e.g., related to the conclusions of the study, major flaws in design, or how it is used in risk assessment]

Resolution:
[Provide a description of the resolution if there is a discrepancy between the primary and the secondary reviewer]

11. References:

SAS Institute. 2010. JMP Pro 9.0.2, SAS Institute, Cary NC.

SAS Institute. 2010. SAS Enterprise Guide 4.3, SAS Institute, Cary, NC.

Scholer, J. and V. Krischik. 2014. Chronic Exposure of Imidacloprid and Clothianidin Reduce Queen Survival, Foraging, and Nectar Storing in Colonies of *Bombus impatiens*.

Primary Reviewer (EPA): Michael Wagman, Biologist
Environmental Risk Branch VI
Environmental Fate and Effects Division

Date: 11/27/14

Secondary Reviewer (PMRA):
Date: